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THE EFFECT OF THE CULTURE MEDIUM ON THE AGGLUTINATIVE PROPERTIES OF BACILLUS PARATYPHOSUS B.

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Theoretically, differences in the metabolism of bacteria may affect agglutination in two ways: Either the agglutinogenic substance or the agglutinable substance may be changed. If the agglutinogenic substance be diminished, injection of the bacteria into animals will cause a lowered production of agglutinins; if increased, a serum of higher titer. As a result of diminishing the agglutinable substance, the organisms will not agglutinate even in the presence of a serum of high titer; increasing the agglutinable substance results in the agglutination of the organisms even by a serum poor in agglutinins. The specificity of the agglutination may also be affected.

Many observations have been made on variations in agglutinations due to the growth of bacteria on blood agar or serum broth. Kirstein[1] found that typhoid bacilli, when grown in serum broth, were slightly less readily agglutinated than when grown in ordinary broth. Bordet and Sleeswijk[2] noted that the serum of rabbits inoculated with a plain agar strain of B. pertussis would agglutinate only that strain, while the serum of rabbits immunized with organisms grown on hemoglobin agar would agglutinate both strains. Later Bordet[3] observed that the blood agar strain, if kept for a few years, would not agglutinate with the serum of a rabbit immunized with a freshly isolated strain; in other words, the artificial cultivation of this strain on blood agar brought about the same change in a few years that was noticed on plain agar in a few days. The agglutinable substance in typhoid bacilli is decreased by growth on blood agar according to Sick.[4] The most striking results were obtained by Gay and Claypole[5] who, in working with B. typhosus, found that two successive transfers on 10% rabbit blood agar produced a strain whose serum would agglutinate its own culture and the plain agar strain indiscriminately, but which could not be agglutinated by the serum of a rabbit immunized with the plain agar strain. They advise, therefore, using animals immunized with the blood agar strain for the purpose of diagnosis. Bull and Pritchett,[6] however, were unable to confirm Gay and Claypole's observations altho they made 25 successive transfers on 10% blood agar of 57 strains of typhoid bacilli.

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[1] Ztschr. f. Hyg., u. Infektionskr., 1904, 46, p. 229.

[2] Ann. de l'Inst. Pasteur, 1910, 24, p. 476.

[3] Jour. State Med., 1913, 21, p. 513.

[4] Centralbl. f. Bakteriol., I, O., 1912, 64, p. 111.

[5] Arch. Int. Med., 1913, 12, p. 621.

[6] Jour. Exper. Med., 1916, 24, p. 35.

Lessened agglutinability in cultures of B. typhosus freshly isolated from the body has been commonly noted, yet Porges and Prantschoff[7] injected a suspension of living typhoid bacilli into the ear vein of a rabbit and found, when they isolated the organism from the gallbladder 35 days later, that it did not agglutinate differently from the original culture which had been kept growing on ordinary media. Sacquépée,[8] however, caused strains of typhoid bacilli to become less readily agglutinated due to growth in collodion sacs in the peritoneal cavities of rats immunized to typhoid. The change was slow, taking 5 months or more and was easily lost by transferring a few times on plain agar. Walker[9] found that cultivation of the typhoid bacillus in its immune serum gave diminished agglutinability, but this decrease was very transitory. Similarly, Moon[10] caused poorly agglutinable strains recently isolated from typhoid patients to have a higher agglutinability by cultivation on agar. Stryker,[11] in studying the reactions of pneumococci, observed that when grown in its immune serum (4 transfers) a pneumococcus showed decreased agglutinability with its homologous serum and less specificity. Normal serum broth and ordinary broth did not, however, affect the reaction of these pneumococci.

The effect of utilizable carbohydrate on bacterial metabolism has been a subject of much interest and possibly, as Kendall[12] has stated, of practical importance. His work showed that the presence of fermentable sugar in considerable amounts in the medium in which bacteria of the colon-typhoid group are grown will reduce the nitrogen content of their bodies one-fifth. Kendall, therefore, concluded that until the exact influence of this variation in nitrogen in terms of immunizing power can be determined, it would be logical to grow bacteria to be used for vaccines in a protein medium free from sugar. Gloessner,[13] in studying the effect of the medium on the agglutinability of bacteria, noted that the presence of sugar had an unfavorable influence on the agglutinogenic properties of B. typhosus and caused a slight decrease in agglutinability. However, as reported by Simonds,[14] the injection of suspensions of killed typhoid bacilli grown for 2 weeks on dextrose agar gave a serum of higher titer than the injection of suspensions of B. typhosus kept on plain agar. He also noted that cultivation on dextrose agar increased the agglutinability of the bacilli. Similarly conflicting observations are made by Sehrwald[15] and Eisenberg[16] concerning the effect of growth of the typhoid, paratyphoid, and dysentery bacilli on potato. The former claimed that the organisms were more readily and rapidly agglutinated than when cultivated on any other medium, but the latter found no variation in the agglutinability of the bacilli.

Changes caused by bactericidal substances have been reported by few observers. Altmann and Rauth[17] by growing B. coli for several generations on "carbol-agar," produced a strain which would not agglutinate with the serum of rabbits immunized with the original strain nor would the serum produced by this "carbol-agar" organism agglutinate the plain agar strain. This variation was relatively permanent, for a month's passage on plain agar did not make it revert to the original reaction. Staphylococci exposed to corrosive sublimate (0.1%) or to phenol (0.75%), according to Abbott,[18] can produce a serum which will agglutinate the normal agar strain in a dilution of 1:1000. However, these organisms can be agglutinated by their own serums no higher than in a dilution of 1:40 and show no trace of clumping with the serum of normal staphylococci. These bacteria regain their former agglutinating power when transferred on normal mediums 5 or 6 times. The observations of Russ and Chambers[19] that diphtheria bacilli clump when exposed to radium emanations make it seem probable that slight variations in the radium content of the culture medium may bring some variability in agglutination.

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[7] Centralbl. f. Bakteriol., I, O., 1906, 41, p. 658.

[8] Ann. de l'Inst. Pasteur, 1901, 15, p. 249.

[9] Jour. Path. and Bacteriol., 1902-3, 8, p. 34.

[10] Jour. Infect. Dis., 1911, 8, p. 463.

[11] Jour. Exper. Med., 1916, 24, p. 49.

[12] Boston Med. and Surg. Jour., 1913, 168, p. 825.

[13] Ztschr. f. exper. Path. u. Therap., 1905, 1, p. 640.

[14] Jour. Infect. Dis., 1915, 17, p. 500.

[15] Deutsch. med. Wchnschr., 1905, 31, p. 261.

[16] Centralbl. f. Bakteriol., I, O., 1906, 41, pp. 752 and 823.

[17] Ztschr. f. Immunitätsforsch., Orig., 1910, 7, p. 629.

That the physical environment may affect the agglutination of the organism is shown by the following observations. Nicolle and Trenel[20] found that cultivating the typhoid bacillus at 42 C. caused a loss in both its agglutinative and agglutinogenic properties. Later Eisenberg[16] caused a reduction in the agglutinability but not in the agglutinogenic properties by growing B. typhosus at this temperature.

With a few exceptions all the observations on this subject agree that variations in the agglutinogenic and agglutinative properties are very difficult to effect and that such changes consist of a slight decrease in the highest dilution to which the organisms are agglutinated rather than the complete loss of the agglutinogenic or agglutinative powers. Such variations are transitory rather than permanent.

The conflicting statements in the literature and the few attempts at producing variations in paratyphoid bacilli made it a matter of interest to study the effect of the medium on the agglutination of B. paratyphosus B. The organism used was a culture isolated from the blood of a paratyphoid fever patient by Irons and Jordan[21] (Para. B. 12). This bacillus has shown constant characteristics during the 5 years it has been kept in stock.

The mediums tested were sugar-free agar as a control medium, 10% rabbit blood agar, Loeffler's serum, 1% dextrose and 1% lactose broth and agar, potato, and Jordan's nonprotein medium to which was added 1.5% agar. The reaction of the sugar mediums and sugar-free agar was 0.5% acid to phenolphthalein. The organism was transferred on these mediums daily for 2 weeks before the first injection into rabbits was made and 44 times before the agglutination tests were tried. To control the factor of individual variations in rabbits, 2 animals of different types were inoculated with each strain of the bacillus.

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[18] Jour. Med. Research, 1912, 26, p. 513.

[19] Proc. Roy Soc. Med., 1911-1912, 5, p. 198, Path. Sect.

[20] Ann. de l'Inst. Pasteur, 1902, 16, p. 562.

[21] Jour. Infect. Dis., 1915, 17, p. 234.

Altho Neisser[22] showed that there is no strict parallelism between the number of bacilli injected and the agglutination value produced thereby, yet it seemed better to standardize the suspensions before injecting them into the rabbits, 100 million bacteria being injected each time. These suspensions were made in sterile physiologic salt solution from 24-hour old cultures of the medium tested except in the case of the potato strain. Since the suspensions made directly from this medium contained so many potato particles, the strain was transferred to agar once before injection. The tables containing my results do not include the original agglutinating value of the rabbits since only 2 agglutinated in a 1:20 dilution, and most did not clump the bacteria at all. The rabbits were immunized by injecting first killed and then live suspensions of the bacteria subcutaneously and intraperitoneally. They were bled 10 days after the third intraperitoneal injection.

The macroscopic method of agglutination was used, each strain being tested not only with its homologous serum, but with each heterologous serum also. Instead of merely adding 10 c c of physiologic salt solution to each slant as is ordinarily done, the suspensions for the agglutination tests were made by washing down cultures and then diluting them to a like density (roughly estimated by the microscopic method of standardizing vaccines), thus avoiding any variation in agglutination due to a difference in the number of bacilli present. To each serum dilution (1 c c) the same amount of suspension was added. The tubes were then incubated at 37 C. for 2 hours, placed in the icebox for 18 hours, and read. To check results I marked the tubes in cipher and did not ascertain their identity until all the results were read and recorded.

Incidentally, it may be observed (1) that the growth on blood agar was almost 3 times as heavy as on ordinary agar and the bacilli showed a pronounced variation in morphology, becoming thicker and larger, more oval shaped, and tending to grow in chains. (2) A similar change was noted on Loeffler's serum, some of the bacilli also forming granules. (3) The growth on the sugar-containing mediums was almost 50% denser than on the sugar-free agar. (4) An effect of potato on paratyphoid bacilli was manifested by the fact that altho the development on potato was slight even in 48 hours, yet return to ordinary agar gave a much heavier growth of bacteria than formerly. (5) At first, cultivation of the organism on nonprotein agar was difficult, but when it had become accustomed to this medium about 60% as many bacteria developed on this agar in 24 hours as on ordinary agar.

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[22] Ehrlich: Collected Studies on Immunity, 1906, Chap. 12.

The following tables show the results obtained from the agglutination tests.

TABLE 1

TESTS OF RABBITS IMMUNIZED WITH STRAIN GROWN ON SUGAR-FREE AGAR

Medium	Rabbit	Control	1/50*	1/250	1/500	1/1000	1/2000	1/5000	1/10000
Sugar-free agar	1	—	+++	+++	++	++	++	++	+
	2	—	+++	++	++	++	+	+	tr.
Blood agar	1	—	++	++	++	+	tr.	tr.	—
	2	—	++	+	+	+	tr.	—	—
Loeffler's serum	1	—	+++	++	++	++	++	+	tr.
	2	—	+++	++	++	+	+	tr.	—
Dextrose	1	—	++	++	++	++	++	+	tr.
	2	—	+++	++	++	++	+	tr.	—
Lactose	1	—	++	++	++	++	+	+	tr.
	2	—	+++	++	++	++	+	tr.	—
Potato	1	—	+++	++	++	++	++	+	tr.
	2	—	+++	++	++	+	+	—	—
Nonprotein	1	—	+++	+++	++	++	+	+	tr.
	2	—	+++	+++	++	++	+	tr.	—

* Explanation: +++ = complete agglutination; deposit and supernatant fluid clear. ++ = Marked agglutination; deposit but supernatant fluid not clear. + = Slight agglutination; flakes visible to the eye. tr. = Trace. — = No agglutination.

TABLE 2

TESTS OF RABBITS IMMUNIZED WITH STRAIN GROWN ON BLOOD AGAR

Medium	Rabbit	Control	1/50	1/250	1/500	1/1000	1/2000	1/5000	1/10000
Sugar-free agar	1	—	++	++	++	++	+	tr.	tr.
	2	—	++	++	++	+	+	+	—
Blood agar	1	—	+++	++	++	++	++	++	tr.
	2	—	+++	++	++	++	++	+	tr.
Loeffler's serum	1	—	++	++	++	+	tr.	—	—
	2	—	+++	++	++	++	+	+	tr.
Dextrose	1	—	++	++	++	++	++	tr.	tr.
	2	—	+++	++	++	++	+	+	tr.
Lactose	1	—	++	++	++	+	tr.	—	—
	2	—	+++	++	++	++	+	+	tr.
Potato	1	—	++	++	++	+	tr.	—	—
	2	—	+++	++	++	++	+	+	tr.
Nonprotein	1	—	+++	++	++	++	+	tr.	—
	2	—	+++	++	++	+	+	tr.	tr.

TABLE 3

TESTS OF RABBITS IMMUNIZED WITH STRAIN GROWN ON LOEFFLER'S SERUM

Medium	Rabbit	Control	1/50	1/250	1/500	1/1000	1/2000	1/5000	1/10000
Sugar-free agar	1	—	+++	++	++	++	+	+	+
	2	—	+++	++	++	+	+	+	tr.
Blood agar	1	—	+++	++	++	++	++	+	+
	2	—	+++	++	++	++	++	+	tr.
Loeffler's serum	1	—	+++	+++	++	++	++	++	+
	2	—	+++	+++	++	++	++	+	+
Dextrose	1	—	+++	++	++	++	++	+	+
	2	—	+++	++	++	++	+	+	+
Lactose	1	—	+++	++	++	++	++	+	+
	2	—	+++	++	++	++	+	+	tr.
Potato	1	—	++	++	++	++	++	+	tr.
	2	—	+++	++	++	++	++	+	—
Nonprotein	1	—	+++	++	++	++	+	+	tr.
	2	—	+++	++	++	++	++	+	tr.

TABLE 4

TESTS OF RABBITS IMMUNIZED WITH STRAIN GROWN ON DEXTROSE BROTH

Medium	Rabbit	Control	1/50	1/250	1/500	1/1000	1/2000	1/5000	1/10000
Sugar-free agar	1	—	++	++	++	++	+	tr.	—
	2	—	+++	++	++	++	++	tr.	—
Blood agar	1	—	++	++	++	+	—	—	—
	2	—	++	++	++	+	+	tr.	—
Loeffler's serum	1	—	+++	++	++	++	++	+	tr.
	2	—	+++	++	++	+	+	tr.	—
Dextrose	1	—	+++	+++	+++	++	++	+	+
	2	—	+++	++	++	++	++	++	+
Lactose	1	—	+++	+++	++	++	++	+	tr.
	2	—	+++	++	++	++	++	+	tr.
Potato	1	—	+++	++	++	++	++	+	tr.
	2	—	+++	++	++	++	+	+	tr.
Nonprotein	1	—	+++	++	++	++	+	+	tr.
	2	—	+++	++	++	+	+	+	tr.

TABLE 5

TESTS OF RABBITS IMMUNIZED WITH STRAIN GROWN ON LACTOSE BROTH

Medium	Rabbit	Control	1/50	1/250	1/500	1/1000	1/2000	1/5000	1/10000
Sugar-free agar......	1	—	+++	++	++	++	++	+	—
	2	—	+++	++	++	++	+	+	tr.
Blood agar...........	1	—	++	++	++	+	+	tr.	—
	2	—	++	++	+	+	+	tr.	—
Loeffler's serum......	1	—	+++	++	++	++	+	+	tr.
	2	—	+++	++	++	++	++	+	tr.
Dextrose.............	1	—	+++	++	++	++	++	+	+
	2	—	+++	++	++	++	++	++	+
Lactose..............	1	—	+++	+++	++	++	++	+	tr.
	2	—	+++	++	++	++	++	+	+
Potato...............	1	—	+++	++	++	++	++	+	tr.
	2	—	+++	++	++	++	++	+	tr.
Nonprotein...........	1	—	+++	++	++	++	+	+	—
	2	—	+++	++	++	++	+	+	tr.

TABLE 6

TEST OF RABBIT IMMUNIZED WITH THE STRAIN GROWN ON POTATO

	Control	1/50	1/250	1/500	1/1000	1/2000	1/5000	1/10000
Sugar-free agar.................	—	+++	++	++	++	++	+	—
Blood agar......................	—	+	+	+	+	tr.	—	—
Loeffler's serum................	—	+++	++	++	++	++	++	+
Dextrose........................	—	+++	++	++	++	++	++	+
Lactose.........................	—	+++	++	++	++	++	+	—
Potato..........................	—	+++	++	++	++	++	+	tr.
Nonprotein......................	—	+++	++	++	++	+	+	+

TABLE 7

TEST OF RABBIT IMMUNIZED WITH THE STRAIN GROWN ON NONPROTEIN AGAR

	Control	1/50	1/250	1/500	1/1000	1/2000	1/5000	1/10000
Sugar-free agar.................	—	+++	++	++	++	++	+	tr.
Blood agar......................	—	++	++	+	+	tr.	—	—
Loeffler's serum................	—	+++	++	++	++	++	+	tr.
Dextrose........................	—	++	++	++	++	++	+	+
Lactose.........................	—	+++	+++	++	++	++	+	tr.
Potato..........................	—	+++	++	++	++	++	+	tr.
Nonprotein......................	—	+++	+++	++	++	++	++	+

TABLE 8

TEST OF RABBIT IMMUNIZED WITH STRAIN GROWN ON PLAIN AGAR, 5 INTRAPERITONEAL INJECTIONS

	Control	1/50	1/250	1/500	1/1000	1/2000	1/5000	1/10000
Sugar-free agar	—	+++	++	++	++	++	++	+
Blood agar	—	++	++	+	+	tr.	tr.	tr.
Loeffler's serum	—	+++	++	++	++	++	++	+
Dextrose	—	+++	++	++	++	++	+	+
Lactose	—	+++	++	++	++	++	++	+
Potato	—	+++	++	++	++	++	++	+
Nonprotein	—	+++	+++	++	++	++	++	+

SUMMARY AND CONCLUSIONS

A typical paratyphoid B bacillus was transferred 44 times on each of the following mediums: Sugar-free agar; 10% rabbit blood agar; Loeffler's serum; 1% dextrose broth; 1% lactose broth; potato, and Jordan's nonprotein medium. Two rabbits were immunized with each strain produced on these mediums. The period of immunization was the same for all the rabbits. In both the injection of the rabbits and the agglutination tests the suspensions of the bacteria used were standardized (approximately 100,000,000 per c c). The following results were obtained:

Each strain, as a rule, agglutinated better with its homologous serum than with any heterologous serum. The serum produced by the potato strain, however, was an exception, since it agglutinated the organisms grown in dextrose broth and on Loeffler's serum better than its own strain.

The variations in agglutination were not marked, consisting merely of slight differences in the highest dilutions to which the strains are agglutinated.

The effect of growth on blood agar was most pronounced, for altho the blood agar strain by no means showed complete loss of agglutinability with heterologous serums, yet it was agglutinated to a far less degree by these serums than by its own, except in the case of the serum of the rabbits immunized with the Loeffler's serum strain. The blood agar strain did not, however, produce a serum of higher agglutinating titer for heterologous strains than did the other strains.

The fact that the strain grown on Loeffler's serum did not show a similar decrease in agglutinability might be explained either by the absence of red blood corpuscles or by the presence of the sugar in the medium.

The strains grown on dextrose and lactose broths produce serums with a relatively high titer for both homologous and heterologous strains of bacteria. But they did not show the marked increase in their agglutinogenic properties noted by Simonds[14] with the typhoid bacillus.

A slight increase in the amount of the agglutinogenic substance produced toward organisms cultivated on other carbohydrate-containing mediums was noted in the potato strain.

The nonprotein agar strain did not show a marked decrease in its agglutinative property such as was observed by Gloessner in his work with B. typhosus.

One rabbit immunized for a longer period of time than the others showed a marked increase in the titer of its serum. Possibly, some changes in agglutination attributed to the increase in the agglutinogenic property of the bacteria may be due to variations in the time element in immunizing the rabbits.